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Identifying Drought Tolerance Traits in Tennessee Soybean Genotypes: Recovery from Severe Water Deficit Stress

Samuel W. Purdom
spurdom1@vols.utk.edu

To the Graduate Council:

I am submitting herewith a thesis written by Samuel W. Purdom entitled "Identifying Drought Tolerance Traits in Tennessee Soybean Genotypes: Recovery from Severe Water Deficit Stress." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant Sciences.

Avat Shekoofa, Major Professor

We have read this thesis and recommend its acceptance:

Vince Pantalone, Liesel Schneider, Lori Duncan, Angela McClure

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

Identifying Drought Tolerance Traits in Tennessee Soybean Genotypes: Recovery from Severe Water Deficit Stress

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ABSTRACT

Ninety-four percent of U.S. grown soybeans are produced under rainfed conditions with intermittent droughts occurring late in the season during reproductive growth stages. Due to the temporary nature of drought, the ability of a crop to survive and recover effectively from water deficit stress is important for ensuring yield stability. In 2019 and 2020, two greenhouse experiments and two field studies were conducted to screen eleven soybean genotypes for transpiration response and recovery from water deficit stress and high vapor pressure deficit (VPD). In the first greenhouse study, soybean plants were grown in pots sealed to prevent evaporation and plants gradually transpired the full amount of water in each pot (dry-down) before being re-watered. In the second controlled environmental study, plants were exposed to three levels of VPD. In both controlled environmental experiments transpiration rate was measured gravimetrically. In the field, portable rainout shelters were used to exclude precipitation from soybean plots while stomatal conductance (g_s), and specific leaf area (SLA) were measured. In the dry-down and field experiments, recovery irrigation was applied after a period of Stage III water deficit stress and leaf wilting score (WS) was rated visually on a scale of zero to five. In the field, pre- and post-recovery canopy temperature (CT) was measured. Genotypic differences in soybean contributed to differentiated response to water deficit and high VPD in both greenhouse and field experiments. In the dry-down experiment, the genotypes TN09-029, TN16-520R1, and Ellis had superior recovery from water deficit stress based on WS while USG Allen and TN09-008 had the highest

transpiration recovery; RIL #1360 and USG 7496XTS showed the least ability to recover from stress. In the field, Ellis, USG Allen, and TN09-029 exhibited a more robust recovery based on WS and Ellis exhibited the highest post-recovery g_s. TN09-029 and Ellis had the largest reduction in CT after recovery. Ellis had the highest yield at 3516.56 kg/ha and consistently expressed a desired response of early decrease in transpiration rate with drying-down, delayed wilting in the field when soil water deficit developed, and had highest stomatal conductance post-recovery under extreme water deficit environments.

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CHAPTER I INTRODUCTION

Review of Literature

Background

Soybean [*Glycine max (L.) Merr*] is one of the most important food crops in the world as a source of vegetable protein, oil, and carbohydrates with 96.6 million metric tons produced in 2019 in the United States alone (USDA 2019). Leng and Hall (2019) demonstrated with an ensemble of eleven crop simulation models that average soybean yields in the United States could decrease by a factor of 15.1% - 16.1% by the end of the 21st century as a result of drought. Zhao et al. (2017) indicated that without effective adaptation, genetic improvement, and CO_2 fertilization each degree-Celsius increase in global mean temperature would, on average, reduce global yields of soybean by 3.1%, wheat (*Triticum aestivum*) by 6.0%, and maize (*Zea mays* L.) by 7.4%. In the United States, the percentage of soybean acreage under irrigation ranged from six to seven percent of the total from 2000 to 2016 (Irwin et al., 2017), highlighting the dependence of soybean production on timely rainfall. In response to continuing change in environmental conditions in soybean producing areas, the development of soybean germplasm that has an increased ability to produce stable yield under highly variable climatic conditions, and more specifically drought, is gaining importance as one aspect of mitigating the impact of climate change on global food production (Dubey et al., 2019).

The current trend towards using genocentric molecular approaches to improving crop yield and response to drought focuses on understanding the regulation of genes that might be relevant in plant development and growth.

However, disconnects have arisen: manipulation of genes to alter a single physiological process does not take into account the complex and often interdependent mechanisms that contribute to a plant's response to stress (Sinclair and Purcell, 2005). Similarly, much of the genocentric research does not consider the need to evaluate enhanced plant performance traits under applied conditions. Sinclair (2011) suggested a top-down approach to crop breeding that utilizes a whole-crop perspective where performance of a plant community, or crop, composed of plants with altered traits can be studied for expression of a desired behavior across a range of environments. This focus on the performance of intact plants and their expression of desired traits is contrary to the genocentric approach, which attempts to generalize some molecular level transformation to the whole crop level, often ignoring interdependent physiological mechanisms. Plant response to water deficit is likely under the control of many genes and interactions with the biotic and abiotic environment (Sinclair, 2011).

Plant Physiological Responses to Soil Drying Cycle

Sinclair and Ludlow (1985) proposed three stages of plant response to soil drying and onset of drought stress. Stage I characterizes the situation in which soil water is plentiful, root uptake is equal to transpiration, and stomata are fully open. In this situation, transpiration rate varies as a function of atmospheric demand (Rosas–Anderson et al., 2014). Plants remain in Stage I until the soil water content declines up to a threshold of the fraction of transpirable soil water (FTSW) (Rosas–Anderson et al., 2014) (Fig. 1; all tables and figures are located in the appendix). When FTSW drops below that threshold, plants enter Stage II of water deficit stress, where stomata begin to close for periods of the day when soil water uptake from the roots cannot meet the full evaporative demand of the atmosphere. Stage II generally begins at an FTSW range of 0.35 to 0.45 depending on interspecific and intraspecific variation in level of stomatal control under drying soil (Ray and Sinclair, 1997; Devi et al., 2009). Stage III stress occurs when the FTSW effectively reaches zero and no further reduction in water loss through stomatal closure can be reached. Stage III may also be defined as the point where the relative transpiration ratio of stressed plants decreases below 0.1 of well-watered plants (Rosas-Anderson et al., 2014). In Stage III, stomata remain closed and rate of transpiration is controlled by the epidermal conductance of the plant. When ability of roots to remove water from the soil drops below the epidermal conductance rate, leaves approach the critical relative water content (RWC_c) and begin to die (James et al., 2008).

Drought Tolerance Mechanisms in Plants

Drought tolerance in plants depends on a complex of morphological and physiological traits that maximize water uptake and minimize water loss such as deep rooting habits, high stomatal control, low epidermal loss of water, and more sensitive regulation of leaf area (Hossain et al., 2014). Genotypic differences in soybean contribute to differences in response to water deficit stress including: epidermal conductance, osmotic potential, RWC (James et al., 2008), slow canopy wilting (Ye et al., 2019), transpiration and photosynthetic compensation

(Gilbert et al., 2011), specific leaf area and water use efficiency coefficient (Shekoofa et al., 2016), nitrogen fixation (Serraj et al., 1999) and, more recently, recovery from severe drought stress (Rosas-Anderson et al., 2021; Rosas-Anderson et al., 2020).

One aspect of drought tolerance is the ability of plants to tolerate dehydration of tissues, meaning that they exhibit a lower RWC_c, and often utilize osmotic adjustment (OA) in order to compensate for lower water potentials that are found in drought-stressed plants (Lawn and Likoswe, 2008). This tolerance strategy allows plants to maintain metabolic activity and net photosynthesis, albeit reduced, under increasing water deficit stress and decreasing tissue RWC (Lawn and Likoswe, 2008). Plants that utilize OA are able to shift the relationship between RWC and water potential by increasing osmotic potential in leaves, allowing them to stay above RWC_c in more extreme water deficit conditions (Sinclair and Ludlow, 1985). Lawn and Likoswe (2008) stated that the rapid mortality and leaf-firing of soybeans under low RWC was partially responsible for the relatively sensitive nature of soybeans to drought as compared to its leguminous relatives such as cowpea (Lawn, 1982). An RWC between 40% and 60% may be lethal in soybean according to James et al. (2008). Therefore, phenotyping for traits that impart increased leaf maintenance in soybean under severe water deficits may lead to the identification of genotypes with a reduced risk of failure and increased productivity potential in areas that experience periodic droughts.

Limited Transpiration and Slow-Wilting

Among the phenotypic responses in soybean to water deficit stress, the delayed canopy wilting trait shows much promise in the identification of drought tolerant genotypes (Sinclair et al., 2010). The slow-wilting trait was first identified in a Japanese cultivar (PI 416937) which also exhibited other physiological differences under water deficit stress as compared to a fast-wilting cultivar, such as lower osmotic potential, higher pressure potential, and higher relative water content (Sloane et al., 1990). Fletcher et al. (2007) found that the slow-wilting trait was associated with lower transpiration rates induced by vapor pressure deficits (VPD) greater than 2.0. Furthermore, the slow-wilting trait in PI 416937 was also shown to be associated with lower stomatal conductance (Tanaka et al., 2010). These factors which contribute to a reduced maximum transpiration rate could be important in rainfed production in regions that experience high VPD conditions by enabling a significant amount of water saving early in the season (Sinclair et al., 2005). The conserved soil water can then be used by the plant later in the season, during reproductive growth stages such as seed fill, when water deficits develop. Crop simulations with soybean show that this trait could result in a greater than 80% increase in yield over much of the United States (Sinclair et al., 2010).

In summary, the slow-wilting phenotype provides an efficient method to evaluate stomatal response of plants to both high VPD and soil water deficit. Commercial cultivars with limited transpiration response to high VPD have been released for maize (Gaffney et al., 2015) and soybean (Carter et al., 2016). While studies have identified genotypic differences in the FTSW threshold at which reductions in transpiration occur in maize (Ray and Sinclair, 1997), sorghum (*Sorghum biocolor L.)* (Gholipoor et al., 2012), and peanut (*Arachis hypogaea* L.*)* (Sinclair et al., 2018), little research has been conducted into FTSW threshold in soybean since Hufstetler et al. (2007) found differences in 23 soybean genotypes grown in sandy soil.

Recovery from Water Deficit Stress

Very few researchers have evaluated the physiological response and recovery of crops from Stage III water deficit stress, most likely due to the fact that the severity of this level of stress on crops in production settings usually results in a crop failure. A study of leaf area maintenance and recovery from drought by Lawn and Likoswe (2008) showed that small genotypic differences in leaf survival during an increasing water deficit can have a large effect on the ability of soybean plants to recover after stress is relieved suggesting that differences in survival and subsequent recovery could have agronomic relevance in terms of preventing crop failure. The same study evaluated genotypes with previously reported drought tolerance in the southern USA (Pantalone et al., 1996) and found that the ability of soybean to survive and recover from a temporary water deficit stress may be more important than efficiency of water uptake during the stress. Genetic variation in recovery of transpiration and leaf maintenance from Stage III stress has been identified in peanut (Rosas-Anderson et al., 2014), and in soybean, where differences in recovery of leaf expansion rates and transpiration were observed in five genotypes in a controlled environment (Rosas-Anderson et al., 2021; Rosas-Anderson et al., 2020). Rosas Anderson et al. (2021) observed that while all soybean genotypes recovered within three days of re-watering, variability in the maximum transpiration rate reached after recovery suggested that genetic differences may confer an advantage during drought conditions.

Thermal Imaging for High-Throughput Phenotyping of Water Deficit Stress Response

In the case of plant water relations, and more specifically, stomatal conductance, transpiration of water from leaves results in a cooling effect due to the latent heat of vaporization and, therefore, a negative correlation between transpiration rate and leaf temperature (Jones et al., 2009). The application of infrared thermography in evaluating plant water status is well reported and reaches several decades in the past (Idso et al., 1981; Jackson, 1982; Gates, 1968). In drought conditions, such as the Stage II and Stage III water deficit stress described above, stomatal closure and the consequent reduction in transpiration rates leads to a measurable increase in canopy temperature relative to the air and to well-watered plants under the same conditions (Casari et al., 2019; Crusiol et al., 2019; Gutierrez et al., 2018).

Screening plants for abiotic stress responses is time-consuming, expensive, and is often not feasible to conduct at a scale that allows for the rapid screening of many genotypes simultaneously (Casari et al., 2019). Thermal infrared (TIR) imaging allows for rapid, non-destructive measurement of large areas. The ability to image many plants or plots at the same time minimizes the issue of variable environmental conditions such as cloud cover and shading that could affect the ability of researchers from spatially phenotyping for responses across genotypes (Crusiol et al., 2019).

TIR technology is being developed for use in a variety of agricultural applications such as determining crop water status and irrigation needs in research as well as commercial settings. Hoffmann et al. (2016) used TIR images to develop water deficit maps for a barley (*Hordeum vulgare)* field for the entire growing season, allowing for precision application and scheduling of irrigation. Sullivan et al. (2007) demonstrated that TIR imagery produced a moderately negative, yet significant correlation ($r = -0.48$, $p = 0.05$) with stomatal conductance and accurately differentiated canopy responses to irrigation treatments in cotton (*Gossypium hirsutum)*. Several studies found that UAS-based TIR imaging was effective in evaluating crop water status and irrigation demand in vineyards (*Vitis vinifera* L.) (Gutierrez et al., 2018; Bellvert et al, 2014), fruit trees (Gonzalez-Dugo et al., 2013), maize (Berni et al., 2009), and sugar beets (*Beta vulgaris)* (Quebrajo et al., 2017). From a phenotyping perspective, Casari et al. (2019) used this technology to compare drought response of different maize hybrids, and differences in canopy temperature between fast-wilting and slow-wilting soybean genotypes grown under different levels of irrigation. An associated increase in yield in low-temperature, slow-wilting genotypes has also been observed (Bai & Purcell, 2018). Comparing the physiological response and canopy temperature of soybean genotypes with differences in temporal water use and leaf maintenance over soil drying and recovery can provide a valuable insight into soybean abiotic stress detection and phenotyping.

Objectives

This study seeks to screen soybean genotypes developed for the mid-south region for drought tolerance traits that will increase sustainability of production in unstable environments. These traits should enable plant breeders to develop cultivars that can provide Mid-South soybean producers with greater yield stability under variable rainfall patterns. This objective can further be broken down into three specific goals: (1) to screen soybean genotypes for early-stomatal closure and delayed-wilting under a progressive water deficit up to and including Stage III, (2) screen soybean genotypes for recovery from prolonged Stage III water deficit stress, and (3) develop and test infrared thermography based phenotyping of soybean physiological responses to drought.

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Appendix

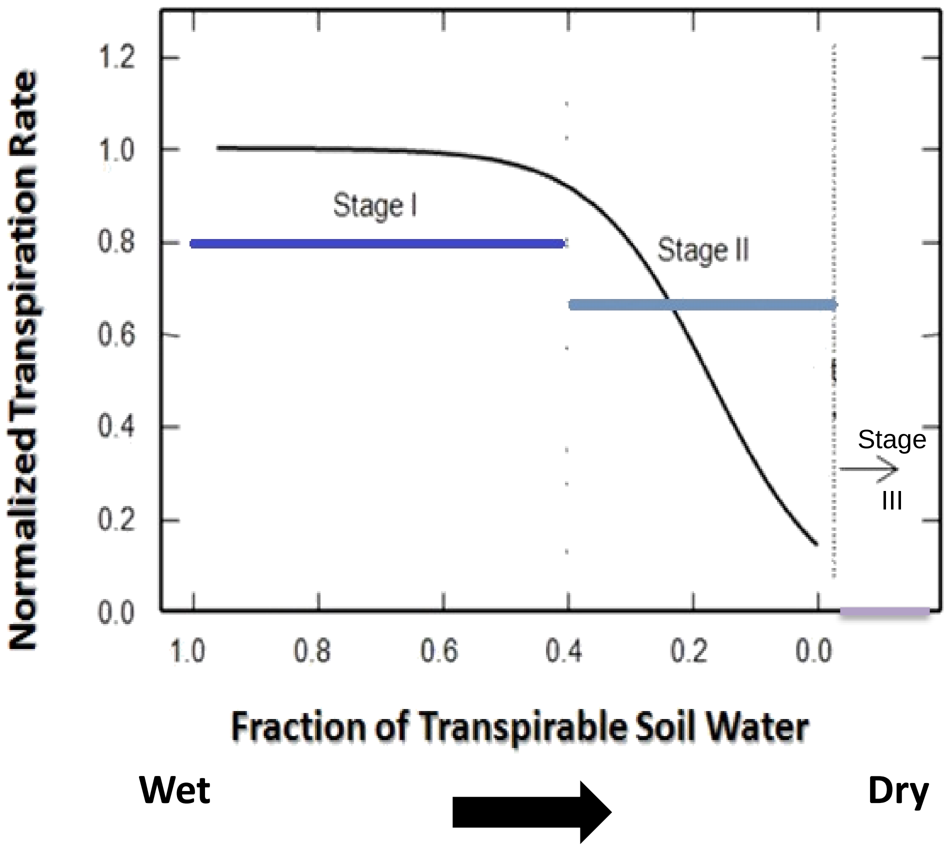

Figure 1. Stage I –Sufficient water is present in the soil, Stage II - Soil begins to dry, plants close stomata for longer periods (often initiated ~ 0.3 - 0.4 FTSW), and Stage III- Further drying, stomata close until water is replenished (Sinclair and Ludlow, 1986; Rosas–Anderson et al., 2014).

CHAPTER II
SOYBEAN RECOVERY FROM STAGE III WATER DEFICIT STRESS AND TRANSPIRATION RESPONSE TO HIGH VAPOR PRESSURE DEFICIT IN CONTROLLED ENVIRONMENTS

Abstract

Soybean, one of the most widely grown row crops in the world and an important source of vegetable protein, is expected to face production challenges with increasing intensity and frequency of drought events. Ninety-four percent of U.S.-grown soybeans are produced under rainfed conditions, with droughts often occurring late in the season during reproductive growth stages. Due to the temporary nature of drought, the ability of a crop to survive and recover effectively from water deficit stress is important for ensuring yield stability. Two greenhouse studies were conducted at the West TN Research and Education Center to determine the transpiration response of eleven Mid-South soybean genotypes to (i) Stage III water deficit stress, (ii) recovery from Stage III stress, and (iii) varying vapor pressure deficit (VPD) levels. In experiment 1 a "dry-down" study was carried out where eight soybean genotypes grown in pots received either the dry-down (DD) or well-watered (WW) treatment. Pots in the DD treatment were allowed to gradually transpire until fraction of transpirable soil water (FTSW) reached zero. A normalized transpiration rate (NTR) was also calculated by dividing the transpiration rates of the DD pots by the WW pots. The FTSW was considered to zero when NTR<0.10. After four days at Stage III stress, DD pots received recovery re-watering, returning them to a well-watered state. Visual wilting score (WS) was also observed during the dry-down and recovery period. In experiment 2, eleven soybean genotypes grown in pots were exposed to three different levels of VPD in a walk-in growth chamber and

transpiration rate (TR) was measured gravimetrically. In experiment 1, genotypes segregated into two groups as FTSW approached zero: fast-wilting and slow-wilting. Five out of eight genotypes tested achieved an NTR greater than 0.50 after four recovery days. Visual wilting score for four out of eight genotypes was reduced to below 1.0 during the recovery period. In experiment 2, two contrasting responses to increasing VPD were observed: seven out of eleven genotypes expressed a two-segment linear response with a VPD breakpoint (BP), where TR began to level off or decrease as VPD increased past a threshold. The remaining genotypes expressed a linear response of increasing TR to VPD. Both the breakpoint response to increasing VPD and superior recovery from Stage III water deficit provide pathways for development of drought tolerant soybean lines.

Introduction

Soybean is an important agronomic crop in Tennessee, with 1.4 million acres planted in 2019 (USDA, 2020). In Tennessee, 95% of soybean acreage in 2019 was produced with no irrigation (Bowling and Smith, 2019, p. 9). Crop models indicating that soybean yield could decrease in the United States by 15.1% to 16.1% by the end of the 21st century as the result of drought (Leng and Hall, 2019) emphasize the need to develop innovative solutions for sustainability in dryland soybean production including Tennessee.

Sinclair (2010) summarized and analyzed the usefulness of five soybean drought tolerance traits which have been the subject of much research. For the most part, research in pursuit of drought-tolerance traits in soybean has focused solely on the physiological response of plants during the development of water deficit stress (Ries et al., 2012; Devi et al., 2014) while few have tried to understand the subsequent recovery of plants after the drought stress is alleviated. In humid climates such as the Mid-South of the United States, periods of drought followed by rainfall are common during the growing season and understanding how soybeans respond to re-wetting and recovery from a water deficit may be just as important as the response during soil water depletion.

Several studies which have endeavored to understand the potential for water deficit stress recovery in soybean genotypes found that genotypic differences existed in the extent of gas exchange recovery (Rosas-Anderson, 2020; Hufstetler et al., 2007) and leaf expansion rates (Rosas-Anderson, 2021) from a short (one-day) period of Stage III stress. However, no study has imposed a multi-day period of Stage III stress on soybean plants and measured the subsequent recovery of physiological function. Phenotyping techniques such as visual wilting evaluation under short- and medium-term drought conditions has been found to correlate with incidence of plant survival under extreme drought stress (Engelbrecht et al., 2007) and has been associated with differences in yield in soybean (Ye et al., 2020). Both Engelbrecht et al. (2007) and Ye et al., (2020) concluded that delayed-wilting was associated with enhanced drought tolerance.

An additional stress-influencing environmental factor is vapor pressure deficit (VPD) which is compounded by limited water and high temperature, which often occur together. Increased VPD results in increased atmospheric demand for water through evapotranspiration (Penman, 1948). Contrary to increasing the rate of soil water depletion, however, high VPD results in stomatal closure in certain soybean genotypes with the limited transpiration (TR_{lim}) trait (Gilbert et al., 2011) which leads to soil water conservation (Sinclair, 2018). In a model which examined the effect of a limited maximum transpiration rate in sorghum (*Sorghum biocolor L.)*, Sinclair et al. (2005) found that simulated yield was increased in dry years and overall yield stability was improved by limiting transpiration during periods of high atmospheric demand.

The objective of these controlled environment studies was to identify key traits in Tennessee soybean genotypes that could contribute to increased drought tolerance, both in response to dry conditions of increased evaporative demand (i.e., > 2.5 kPa VPD) and in recovering from extended periods of Stage III water deficit stress after water becomes available again.

Materials and Methods

Plant Culture

In two controlled environment studies, eight to eleven soybean genotypes were tested for transpiration response to progressive soil drying, recovery from soil water deficit after re-watering, and changes in VPD. In experiment 1, eight soybean genotypes were grown in pots in a greenhouse while leaf wilting and transpiration rate were monitored as the soil progressively dried to a predetermined level and then received recovery re-watering. In experiment 2, eleven genotypes were grown in pots and transpiration rates were measured during exposure to varying levels of VPD from low to high. Detailed information for all tested genotypes is presented in Table 1 (all tables and figures are located in the appendix).

Experiment 1

Eight soybean genotypes (Table 1) were grown in a greenhouse at the West Tennessee Research and Education Center (WTREC) in Jackson, TN from July 2019 to August 2019. Four soybean seeds of each genotype were sown at a depth of two cm in a soil mix composed of fifty percent sand and fifty percent Lexington silt loam (fine-silty, mixed, active, thermic Ultic Hapludalf) in 3.8-liter pots (18 cm x 19 cm) and inoculated with N-Dure™ soybean inoculant

(Verdesian Life Sciences, Cary, NC). Soybean plants were thinned to one plant per pot thirteen days after planting (DAP). Twelve DAP, pots were fertilized with 200 ml of 0.075 %V/V liquid fertilizer (0-10-10, N-P_2O_5-K_2O, GH Inc., Sebastopol, CA) and again with 200 ml of a 0.06%W/V fertilizer solution (24-8-16, N-P_2O_5-K_2O, Scotts Miracle-Gro Products, Inc., Marysville, OH) at 18 and 24 DAP. Temperature and relative humidity in the greenhouse were recorded every five minutes with EL-USB-2-LCD data loggers (Lascar Electronics Ltd., Erie, PA); daily nighttime temperatures averaged 26.5 ^{o}C and daytime temperatures averaged 33.3 ^{o}C. Figure 3 shows daily high temperatures and maximum vapor pressure deficit (VPD) during the experiment. Natural light was supplemented with artificial lighting to maintain a 15-hour day and 9-hour night schedule. Plants were maintained in a well-watered condition during the initial pre-treatment period and kept in a vegetative growth stage by removing flowers daily. Each genotype was represented by eight replicate pots which were split into treatments, well-watered and water deficit, during the drying phase of the experiment.

water deficit Stress (dry-down) and Recovery

When the plants had four to five trifoliate leaves, 28 DAP, the dry down experiment was initiated. On the afternoon before initiation of the drying cycle, pots were over-watered until dripping and allowed to drain overnight. Pots were then placed into two double-bagged 15-liter plastic bags (Wal-Mart Stores, Inc., Bentonville, AR) and secured at the base of the plant with plastic twist ties to prevent evaporation from the soil, following the method described by Shekoofa et al. (2013). A small plastic tube (13-mm-diam. × 126-mm-long) was inserted adjacent to the plant stem to facilitate daily watering (Fig. 2).

Pots were weighed daily between 1200 and 1400 CST to obtain gravimetric water loss through transpiration. After calculating the daily transpiration rate (TR) of each individual plant for three days, plants were assigned to one of two treatments: deficit stressed (DD) or well-watered (WW). Within each genotype, four plants were assigned to the deficit stressed treatment and four were assigned to the well-watered treatment, which served as a reference for calculating the normalized transpiration rate (NTR).

Normalized transpiration rate is a relative transpiration ratio of water deficit-stressed plants to well-watered plants normalized for plant size and environmental conditions (Shekoofa et al., 2013). A transpiration ratio was calculated daily for each plant by dividing its daily loss in weight by the mean weight loss of well-watered plants of the same genotype (first normalization). Then, the NTR was calculated by dividing each plant’s daily transpiration ratio by the average transpiration rate of the same plant for the first three days of the experiment when all plants were still under well-watered conditions (second normalization). The first normalization was to minimize the influence of large variations in daily TR across days. The second normalization was done to account for plant to plant variation in size, and therefore transpiration ratio among individual plants. This normalization was conducted following the method described by Rosas Anderson et al. (2014).

After daily weighing of plants, water was added to maintain the WW plants within 200 ml of pot capacity, based on the initial pot weight that was established at the beginning of the experiment. Soil water was allowed to gradually transpire at a rate of no greater than 100 ml day^{-1} from the DD plants in order to simulate a prolonged development of water deficit stress. Water was only added to the DD plants if daily water loss exceeded 100 ml day^{-1}. The dry-down continued until all DD plants reached Stage III drought stress, which was when the NTR of each plant dropped below 0.11. The length of time to reach Stage III varied between 36-48 DAP and each DD plant was allowed to remain in Stage III stress for four days.

On the fifth day of Stage III drought stress, DD plants received 300 ml of recovery watering to return the pots to a well-watered state. The plants were maintained in a well-watered state, within 200 ml of pot capacity, by weighing pots every day and adding water as necessary for a four-day recovery period. Recovery of transpiration rate (calculated as NTR) after Stage III drought stress was measured.

Leaf Visual Wilting Score

Each soybean plant was visually assessed for wilting and assigned a score based on severity of wilting, (0-5: 0= no wilting, 1 = a few leaves wilted, 2 = half of leaves wilted, 3 = most leaves wilted, 4= plant severely wilted, 5 = plant dead). The leaf visual wilting score was collected each day for the DD plants during the dry-down and recovery periods.

Experiment 2

In a second controlled environmental study, the response of transpiration rate (TR) to varying vapor pressure deficit (VPD) was investigated in eleven genotypes. This study included the eight genotypes from Experiment 1, two additional genotypes 5002T (Pantalone et al., 2004) and 5601T (Pantalone et al., 2003), parents of existing “Ellis” genotype (Pantalone et al., 2017), and TN Exp TN13-4508R2.

Transpiration Response to High Vapor Pressure Deficit (VPD)

Soybean plants were grown in pots constructed from polyvinyl chloride pipe (10-cm diameter and 20-cm long). The plants were germinated from seeds and grown in a greenhouse at WTREC, Jackson, TN. The bottom of each pot was fitted with a flat end cap, in which a small hole was drilled to allow drainage of excess water. A toilet flange was attached to the top of the pot to allow easy attachment of a VPD chamber during measurements (Fletcher et al., 2007; Shekoofa et al., 2015; Sheldon et al., 2021). Five seeds per pot were sown into commercially available Miracle-Gro potting mix and were inoculated with N-Dure™ soybean inoculant (Verdesian Life Sciences, Cary, NC).

When plants were 4 to 5 weeks old, they were transferred to a walk-in growth chamber approximately two days before starting the measurements. The evening before initiating the experiment, plants were overwatered until water began dripping from the bottom of each pot. Aluminum foil was placed around the base of each plant to cover the soil and prevent evaporation of moisture from the soil. Pots were then allowed to drain overnight (Shekoofa et al., 2015).

To construct each VPD chamber, a 340-mm diameter food container lid (Cambro Manufacturing, Huntington Beach, CA), with the center cut out, was attached to the toilet flange at the top of the pot. The following morning, the aboveground parts of each plant were enclosed in a 21-L clear plastic food container (Cambro Manufacturing, Huntington Beach, CA) by placing the inverted container over the plant and attaching it to the previously installed lid. Each VPD chamber was fitted with a 12-V, 76-mm-diameter computer box fan (Northern Tool and Equipment, Burnsvillle, MN) to continuously stir the air inside the chamber. In addition, a temperature/humidity data logger (MicroDaq, Contoocook, NH) was mounted through the sidewall of each container to measure the chamber environment (Sheldon et al., 2021).

Different levels of VPD were achieved using air flowmeters with either dehumidified or ambient air. After the plant was exposed to each of three levels of VPD, the TR was measured. The temperature was maintained at a constant 32 ^{o}C in the growth chamber. During each day of the experiment, plants were exposed first to low VPD (0.5-1.5 kPa), then medium VPD (1.5-2.5 kPa), and finally high VPD (2.5-3.5 kPa). This sequence was used to avoid any recovery that might be needed if stomatal closure was induced by exposure to the high-VPD treatment.

At each VPD level, after the target was attained, the chamber was allowed to stabilize for 30 minutes, and then the entire pot chamber system was weighed to the nearest 0.1 g to obtain initial weight. The plants were exposed for one hour to each VPD level and weighed at the end of each hour to obtain the final weight. Transpiration rate at each VPD level was calculated as the difference between the initial weight and the final weight. After completing the measurements, leaves were destructively harvested and the total plant leaf area was measured using a leaf area meter (LI-3100, Li-Cor, Lincoln, NE). This enabled the calculation of TR as an expression of water loss divided by plant leaf area (Sheldon et al., 2021).

Data Analysis

Average daily NTR differences between genotypes during recovery, genotype means averaged for the four-day recovery period, and wilting scores averaged for the entire four-day recovery period were compared using a repeated-measures analysis of variance (ANOVA) and mean separation was conducted using Tukey's honest significant difference (HSD) test at an alpha level of 0.05. During dry-down, visual wilting score was calculated for each genotype as the average of the DD plants on each day. Genotype averages for each day during the period when FTSW was less than or equal to 0.30 were calculated and analysis of covariance (ANCOVA) was used to compare slopes of the visual wilting response to decreasing FTSW across genotypes. Fraction of transpirable soil water (FTSW) was calculated by subtracting the cumulative water loss from the initial pot weight and dividing by initial pot weight. All statistical analyses with exception of the two-segment linear regression were performed in JMP 14.2 (SAS Institute, Cary, NC). For the VPD growth chamber experiment, data were analyzed using a two-segment linear regression (Prism 8.0, GraphPad Software Inc., San Diego, CA) for TR vs. VPD. When the slopes for the segments were not significantly different ($p<0.05$), a simple linear regression was used. The two-segment response indicated the VPD breakpoint (BP), or the VPD (kPa) level at which plants begin to close stomata, for each genotype, as well as the slope of each segment. Data from the two measurement days for all plants of a genotype were combined to perform a two-segment or a simple linear regression for TR vs. VPD.

Results

Response to Drying Soil

Soybean plant response to progressive soil drying followed the typical two-segment response curve reported in other studies (Devi et al., 2009; Shekoofa et al., 2013; Sheldon et al., 2021) (Figure 4). The genotypes segregated into two groups when measured by the rate of increase in wilting as FTSW decreased from 0.30 to zero (Table 2). A slower rate of increase in wilting severity was observed in TN09-029, Ellis, TN08-101, and USG Allen, where the slope ranged from -8.3 to -10.1, while a faster rate of wilting relative to FTSW decline was observed in TN16-520R1, TN09-008, USG 7496XTS, and RIL #1360 which ranged from -12.9 to -13.3 (Table 2). There were no statistically significant differences among genotypes in the rate of increase in wilting as FTSW approached zero, however, a clear differentiation in the two patterns of wilting rate was observed. Overall, severe wilting was observed with all genotypes as plants reached Stage III in the controlled environment.

Recovery of Normalized Transpiration Rate

After recovery re-watering was applied to soybean plants on the fifth day of Stage III stress, NTR values increased on day one of recovery to above 0.10 in all genotypes except TN16-520R1 and USG 7496XTS which remained at 0.08 and 0.07, respectively (Table 3). On day two of recovery, all genotypes had recovered from Stage III stress, and by day four of recovery, NTR values ranged from a low of 0.30 in USG 7496XTS to a high of 0.59 in USG Allen.

All genotypes recovered to an NTR greater than 0.50 after four days with the exception of TN08-101 (0.40), TN16-520R1 (0.41), and USG 7496XTS (0.30). Repeated measures analysis of variance (ANOVA) of NTR between genotypes over days 1-4 of the recovery period showed that there was a significant difference between genotypes ($p<.0001$) but no interaction of the genotype and day variables (Table 3).

This indicates that the rates of increase in NTR over recovery between genotypes were similar in slope, but different in magnitude (Figure 5). Significant differences in the NTR average of each genotype for the entire recovery period after Stage III was detected (Table 3). USG Allen had a significantly higher NTR than USG 7496XTS (0.42 and 0.20, respectively, $p=0.008$). However, no significant differences in NTR on each of the individual four recovery days was found at the 0.05 alpha level among genotypes (Table 3).

Recovery of Leaf Maintenance

Plants were visually assessed for wilting rate. The visual scoring was done to evaluate the leaf performance based on approximate damage estimates after re-watering and recovery from Stage III. Average visual wilting score for each genotype on the first day of recovery ranged from 0.1 in TN09-029 and Ellis to 1.63 in USG 7496XTS, and a repeated measures ANOVA for the four-day recovery period indicated a significant effect of genotype (p=0.05). A significant difference (p=0.001) between genotypes was observed for visual scores averaged over the entire recovery period (Figure 6); TN09-029, TN16-520R1, and Ellis had significantly lower average wilting scores (0.08, 0.10, and 0.15, respectively) than RIL #1360 (1.53). No significant differences among genotypes were detected on any single day of the recovery period.

Transpiration Response to High Vapor Pressure Deficit (VPD)

Seven of the eleven soybean genotypes expressed the limited transpiration trait, or early stomatal closure under increased VPD, with VPD breakpoints ranging from 1.8 to 2.7 kPa (Table 4). The four remaining genotypes expressed a linear response to increasing VPD (VPD >2.5 kPa) at the three VPD levels tested. The R^2 values for the genotypes that fit the two-segment linear regression ranged from 0.60 to 0.92. Genotypes Ellis and USG Allen that had quick NTR recovery rates after Stage III of water deficit stress in dry down experiment (Table 3) also had 2.7 and 2.1 kPa VPD BPs, respectively (Table 4). The genotype USG 7496 XTS that had the slowest NTR recovery rates after

Stage III of water deficit stress in dry-down experiment had a linear TR response to increasing VPD (Table 4).

Discussion

Recovery from water deficit stress could play an important role in the performance of crops grown in humid regions of the United States due to the periodic nature of drought and rainfall. Previous studies have investigated drought tolerance traits in soybean, but with a focus on the period of soil water deficit development (Sinclair et al., 2010; Ries et al., 2012; Devi et al., 2014). Recent studies of North Carolina soybean genotypes indicate that ability to recover from drought differs among genotypes and may provide a new pathway for identifying traits in soybean that lead to enhanced drought tolerance (Rosas-Anderson et al., 2021; Rosas-Anderson et al., 2020). While these studies simulated a drying period and recovery, the effects of a prolonged period of Stage III stress was not studied.

This study sought to evaluate each soybean plant response during development of water deficit in the soil (dry-down) and re-wetting (recovery from Stage III) to assess whether any of the tested soybean genotypes can recover from Stage III of water deficit stress. Ideally, a genotype possessing early stomatal closure and slow-wilting traits under soil drying, as well as superior recovery of transpiration and leaf maintenance would be expected to outperform genotypes without these traits in years with less than average rainfall.

Transpiration Response to Dry-down (dry soil) and high VPD (dry air)

The NTR response to decreasing FTSW of all the genotypes tested fit the two-segment linear regression (Fig. 4) with constant transpiration until soil water content decreased below an FTSW threshold. Genotypes Ellis, TN09-029, and TN08-101, which expressed the lowest wilting scores during recovery, were also among those in the slow wilting rate group of genotypes identified during the dry-down.

Interestingly, four genotypes (Ellis, USG Allen, TN08-101, and TN09-0.29) which expressed slow-wilting as FTSW dropped below 0.30 in the soil-drying experiment (Table 2) all expressed limited transpiration under high VPD as well (Table 4). Genotypes which expressed more rapid wilting at low FTSW, TN16-520R1 and USG 7496XTS, fit a linear transpiration response to increasing VPD. This finding confirms the observations of Devi et al. (2015) who found that the slow-wilting trait in soybean genotype PI 416937 was also associated with a limited transpiration rate when VPD increased above 2.0 kPa.

Recovery

One of the most interesting results from this study is the difference in maximum NTR recovery among genotypes. A similar study (Rosas-Anderson et al., 2020) which evaluated recovery of five North Carolina soybean genotypes found that the maximum transpiration rate reached a plateau after just three days of recovery, and a study of cowpea recovery from drought stress found that plants reached a stable maximum transpiration rate after two days of recovery

(Manandhar et al., 2017). However, our current study indicates that the maximum transpiration rate (NTR = 1.0) in soybean was not reached after four days and that, based on an extrapolation of the slopes during the recovery period, NTR could have continued to increase past the four-day mark (Fig. 5).

The range of recovery across soybean genotypes in the current study, from about 30% to over 50% (Table 3), is consistent with the results in a similar study (Hufstetler et al., 2007) which measured NTR for two days of recovery following one day of near Stage III stress. The longer recovery time observed in our study indicates that the prolonged four-day Stage III stress impacted the ability of the plants to fully recover the capacity for transpiration within four days and may have permanently prevented a full recovery to pre-stress levels of transpiration.

Visually rating for wilting after re-watering from Stage III water deficit stress provided a practical way to evaluate soybean leaf maintenance. The comparison of the NTR and visual wilting score observations during the recovery period also determined that only two genotypes, Ellis and TN16-520R1, reached an NTR of greater than 0.50 (50% recovery) and remained among the lowest (best leaf maintenance recovery) in visual rating as well (Table 3; Fig. 6). These two genotypes share a pedigree as TN16-520R1 is a glyphosate resistant backcross derived selection of Ellis (UTIA, 2018, p. 11). This finding points to the presence of common genetic material responsible for the superior recovery mechanisms in these two genotypes. Conversely, among evaluated genotypes, USG 7496XTS was observed to have the lowest NTR and highest visual wilting rating after four days of recovery (Table 3; Fig. 6), suggesting that this genotype was more adversely affected by the Stage III water deficit stress than the other genotypes (Engelbrecht et al., 2007).

The visual wilting score observed over the recovery period (Fig. 6) was influenced by the mortality of plants of certain genotypes during the Stage III stress. Genotypes USG Allen, TN09-008, USG 7496XTS, and RIL #1360 all experienced mortality in one replication during the Stage III stress and/or recovery. Since the rating scale (5=plant dead) accounted for plant mortality, these individuals were included in the analysis and represented an extreme response to the stress.

Further study which applies differing durations of stress as well as an extended recovery period would be useful in establishing a clearer pattern of transpiration rate recovery from severe water deficit stress (i.e., Stage III). A slower rate of soil drying could also help to identify better differentiation in wilting rate between genotypes. Genotypes that expressed desirable responses in more than one trait (Ellis, TN16-520R1, USG Allen) should be included in future studies of drought tolerance traits.

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Appendix

Table 1. All soybean genotypes tested in the greenhouse and field experiments are listed. For each genotype origin, release year, characteristics, maturity and reference are provided in this table.

Genotype	Origin	Release year	Characteristics	Maturity	Reference
TN09-029	University of Tennessee Agricultural Research	N/A	Conventional	IV	University of Tennessee Ag Research
TN09-008 (GoSoy 53C16)	University of Tennessee Agricultural Research	2017	Soybean cyst nematode (SCN) resistant, conventional	V	Pantalone et al., 2018
Ellis	University of Tennessee Agricultural Research	2013	High soymeal protein, semi-bushy, medium-tall, conventional	IV	Pantalone et al., 2017
TN16-520R1	University of Tennessee Agricultural Research	2018	Glyphosate resistant	V	University of Tennessee Ag Research
TN08-101	University of Tennessee Agricultural Research	N/A	N/A	IV	University of Tennessee Ag Research
RIL# 1360	University of Missouri	N/A	Conventional	V	University of Missouri
USG ALLEN	University of Tennessee Agricultural Research	2006	Semi-bushy, glyphosate resistant	V	Pantalone et al., 2010
TN13-4508R2*	University of Tennessee Agricultural Research	N/A	Glyphosate resistant	IV	University of Tennessee Ag Research
USG 7496XTS	UniSouth Genetics, Inc.	2015	Semi-bushy, medium-tall, glyphosate resistant	IV	UniSouth Genetics, Inc.
5601T**	University of Tennessee Agricultural Research	N/A	2001	IV	Pantalone et al., 2003
5002T**	University of Tennessee Agricultural Research	N/A	2002	IV	Pantalone et al., 2004

*Not included in controlled environment experiment 1

**Only included in controlled environment experiment 2

Table 2. Slopes of regression lines for wilting response to FTSW as FTSW decreased from 0.30 to 0.

Genotype	Group Mean	95% Confidence Interval
Ellis	-8.29552	-14.96 to -6.98
USG Allen	-8.65947	-15.40 to - 6.54
TN08-101	-9.49362	-14.84 to -7.10
TN09-029	-10.0818	-15.08 to -6.86
RIL #1360	-12.929	-15.84 to - 6.10
USG 7496XTS	-13.2082	-16.22 to - 5.72
TN09-008	-13.2325	-15.01 to - 6.93
TN16-520R1	-13.3224	-15.31 to -6.63

Table 3. Normalized transpiration rate for 8 soybean genotypes over the four days of recovery from Stage III water deficit stress. Means followed by the same letter do not differ significantly (Tukey's HSD, p=0.05). P-values represent ANOVA results for among genotype differences on each day.

	Normalized Transpiration Rate				
Genotype	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**4-Day AVG**
USG Allen	0.18	0.40	0.53	0.59	0.42a
TN09-008	0.14	0.37	0.53	0.56	0.40ab
TN09-029	0.20	0.39	0.46	0.50	0.39ab
RIL #1360	0.18	0.31	0.40	0.53	0.35ab
Ellis	0.17	0.34	0.38	0.52	0.35ab
TN08-101	0.13	0.28	0.35	0.40	0.29ab
TN16-520R1	0.08	0.23	0.34	0.41	0.26ab
USG 7496XTS	0.07	0.15	0.27	0.30	0.2b
***p*-value**	0.248	0.173	0.174	0.239	0.008

Table 4. Transpiration response of TN soybean genotypes to vapor pressure deficit (VPD) under 32°C in controlled environment. Results from two-segment linear regression include Breakpoint (BP) (X_0) ±SE, Slope 1 (± SE), Slope 2 (± SE), 95% Confidence Interval (CI) of the BP (X_0), and their R^2.

	32°C				
Genotypes	**BP (X_0) ±SE**	**Slope 1 ± SE**	**Slope 2 ± SE**	**R^2**	**95% CI of BP (X_0)**
Ellis	2.7±0.2	47.3±6.8	-34.8±39.9	0.60	2.24 to 3.06
USG Allen	2.1±1.4	24.5±6.3	17.4±10.6	0.80	-1.10 to 5.30
TN09-029	1.8±1.8	29.3±14.3	19.3±6.3	0.91	-2.15 to 5.91
TN08-101	1.9±5.1	34.5±21.8	30.2±7.5	0.88	-9.10 to 12.9
TN16-520R1	Linear	60.9 ± 10.0	-	0.70	-
TN Exp TN13-4508R2	Linear	36.3±5.5	-	0.72	-
TN09-008	2.4±1.1	33.3±3.9	23.0±28.2	0.92	-0.02 to 4.9
RIL #1360	1.9±3.3	34.3±12.6	29.2±26.4	0.64	-5.20 to 9.27
5002T (Ellis parent)	1.8±1.3	32.4±22.0	12.1±6.1	0.73	-0.87 to 4.63
5601T (Ellis parent)	Linear	53.2 ± 8.1	-	0.73	-
USG 7496 XTS	Linear	50.6±12.7	-	0.50	-

Figure 2. Soybean plants with pots enclosed in bags to prevent evaporation from the soil. The red arrow shows watering tube close to the base of the plant.

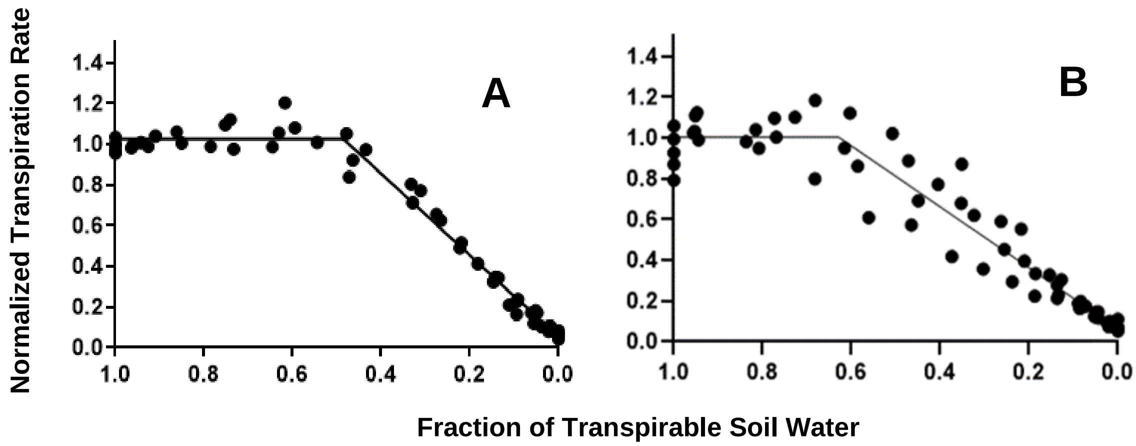

Figure 3. Two segment linear regression equation for normalized transpiration rate (NTR) with decline in fraction of transpirable soil water (FTSW) for a soybean line without early stomatal closure (RIL #1360, A) and a line with early stomatal closure (Ellis, B).

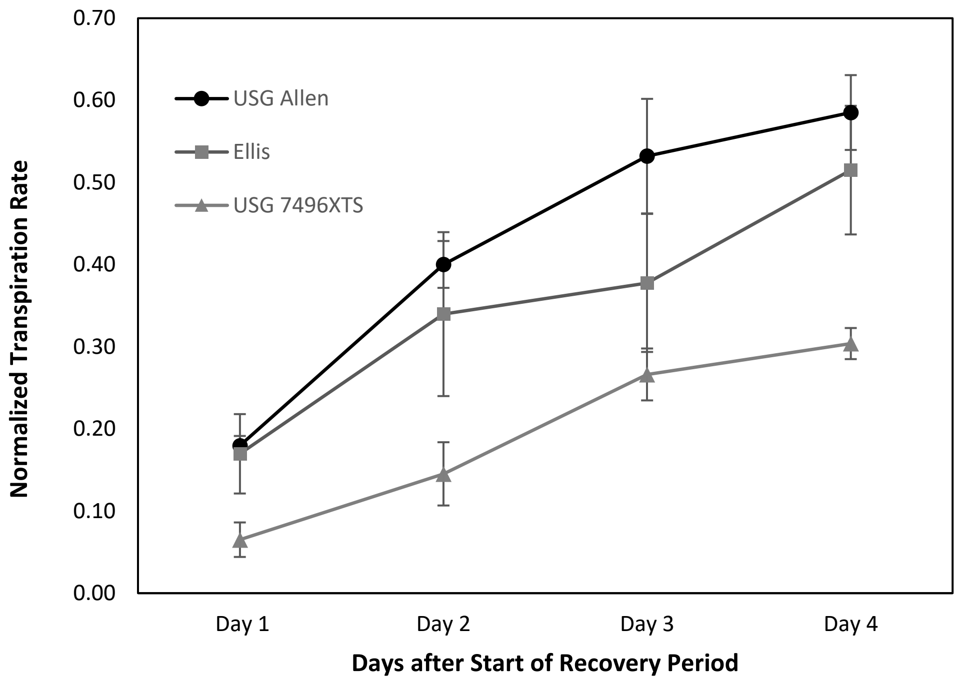

Figure 4. Increase in NTR over time during recovery of three genotypes which represent the highest, lowest, and median of all genotypes in average recovery NTR. Error bars represent standard error of the mean.

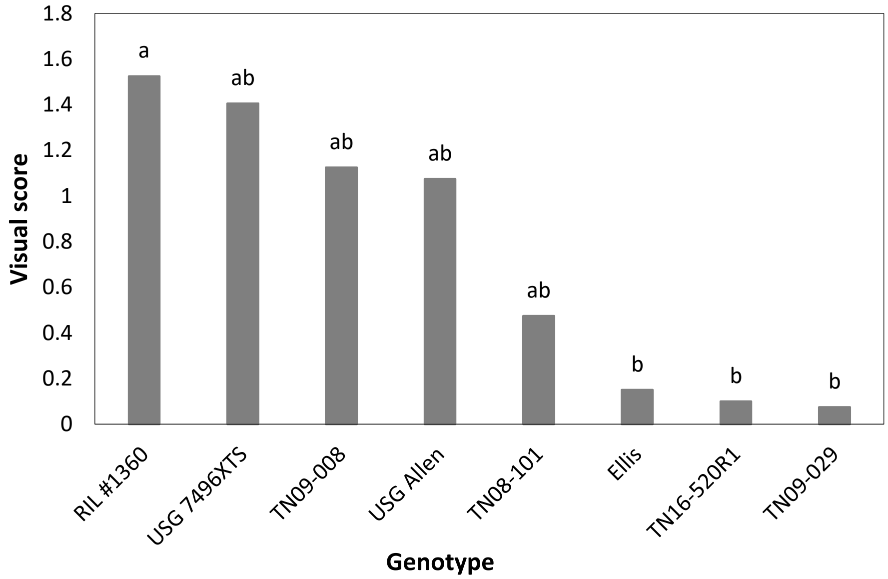

Figure 5. Average visual wilting score during recovery period for all genotypes. Columns accompanied by the same letter are not significantly different (Tukey's HSD, p=0.05).

CHAPTER III
SCREENING TENNESSEE SOYBEAN GENOTYPES FOR RECOVERY FROM A WATER DEFICIT STRESS TREATMENT IN THE FIELD

Abstract

Sensitivity of soybean yield to drought is increasing in the central and southeastern United States. Recovery from drought stress in addition to effective water use are two pathways for improving drought tolerance in soybean. Nine soybean genotypes were grown in two field studies in Jackson, TN in 2019 and 2020. Portable rainout shelters, which excluded all rainfall and irrigation, were imposed over the soybean plots at 80% canopy closure until Stage III water deficit stress was achieved. Then, recovery irrigation of 38 mm was applied when the majority of soybean plots had reached a state of severe wilting. Stomatal conductance (g_s), visual wilting score (WS), and specific leaf area (SLA) were recorded in each plot from the onset of soil water deficit until 7 days after recovery irrigation. A g_s ratio was calculated to compare g_s during water deficit and recovery to the g_s of the same plot in a well-watered condition. Trends over both years showed that genotypes Ellis and TN16-520R1 had the lowest WS at Stage III stress, while Ellis, USG Allen, TN16-520R1, and TN09-029 had the lowest WS one day after recovery. Ellis most consistently had the highest g_s ratio at Stage III and 1-day recovery in both years. Canopy temperature (CT) measurements of the plots before and after recovery was associated with a change in g_s ratio with larger reductions in CT after recovery in genotypes with larger g_s ratios. Differences among genotypes in SLA before and after recovery varied between years; environmental factors such as very high vapor pressure deficit (VPD) in 2019 during Stage III and recovery periods introduced changes in

plant responses to soil water deficit. Yield data for both years confirmed that Ellis (3516.56 kg/ha) outperformed other genotypes under water deficit, followed by TN16-520R1 (3256.97 kg/ha), USG Allen (3238.1 kg/ha), TN08-101 (3232.76 kg/ha), and TN09-008 (3015.54 kg/ha). Remaining genotypes were significantly (p=0.05) lower in yield than Ellis. This study confirms findings from the controlled environment that transpiration and leaf maintenance responses to drought stress in genotypes Ellis, TN16-520R1, and USG Allen. Physiological responses in the field condition support results from the controlled environment and the desired responses were associated with an increase in yield under the drought stress condition.

Introduction

Water deficit is one of the most critical environmental factors affecting soybean yield in rainfed production (Jumrani & Bhatia, 2018). With increasing sensitivity of yield to drought in soybeans in the central and southeastern U.S. (Zipper et al., 2016), breeding for drought tolerance has become a focus of public soybean breeding programs. One of the major challenges to soybean breeders is overcoming the limited genetic base of soybean (Carter, 2004) and identifying genotypic variation in the desired traits.

While the focus of many research studies has been directed to the response of plants during the depletion of soil water stress (Ries et al., 2012; Devi et al., 2014), the importance of soybean plants ability to recover from a water deficit stress is not to be overlooked. In the humid climate of the Mid-South region of the United States, the periodic nature of droughts followed by rainfall creates a situation where a quick and robust recovery from a period of water deficit stress can provide an agronomic advantage. Recent controlled environmental studies observed genotypic variation in recovery of North Carolina soybean lines from a short period of Stage III water deficit stress (Rosas-Anderson et al., 2021; Rosas-Anderson et al, 2020).

Studies evaluating crop response to abiotic stress typically utilize controlled environmental studies and usage of rainout shelters to simulate drought conditions in field experiments (Jumrani & Bhatia, 2019; Rosas-Anderson et al., 2014). Another technique which may help with phenotyping the soybean plant’s response to water deficit is thermal infrared imaging of the plant canopy. Plant transpiration rate and leaf temperature are negatively correlated due to the effect of evaporative cooling (Jones et al., 2009) and canopy temperature provides a pathway for the rapid non-destructive screening of many genotypes in-situ (Casari et al., 2019).

Several different leaf-scale responses to water deficit stress have been proposed as potential mechanisms of drought tolerance in crop plants. Delayed wilting is one such trait that has been associated with increased yield under drought stress (Ye et al., 2020). A second trait, decreased specific leaf area (SLA) under drought stress, has not been studied in soybean but has been correlated with increased drought resistance in peanut (*Arachis hypogaea* L.) (Songsri et al., 2009; Shekoofa et al., 2016).

The objective of this study was to screen soybean genotypes for responses in leaf maintenance and transpiration rate during the development of Stage III water deficit, and after recovery from the Stage III stress by observing wilting, specific leaf area (SLA), stomatal conductance, and canopy temperature. The study was conducted in the field to simulate applied environmental conditions and to confirm the results of plant recovery observed in the greenhouse (Chapter II).

Materials and Methods

Plant Culture

Two field experiments were conducted at the West Tennessee Research and Education Center (WTREC) to evaluate the stomatal conductance recovery of nine soybean genotypes after re-watering from water deficit stress conditions. The genotypes were selected based on their wide range of responses to re-watering in a greenhouse study (Table 1; all tables and figures are located in the appendix).

The field trials were arranged in a split-plot design where main plots received one of two irrigation treatments: 1) plants were irrigated during the growth period and, 2) plants were covered with portable rainout-shelters after an initial period of vegetative growth. Amounts and times of rainfall and irrigation applications are described in Table 5. Each main plot contained four blocks with nine subplots which were sown with one genotype, at a depth of 3.5 cm, in four, 3.4 m rows, spaced at 76 cm, with a planting density of about 350,000 seeds per hectare. University of Tennessee Extension recommendations for herbicide and pesticide applications were followed as necessary.

The field was planted on May 6 and June 3 in 2019 and 2020, respectively; in 2020, severe deer browsing and damage to seedlings necessitated replanting, resulting in a later soybean planting date compared to 2019. The soil type at the study location was a Lexington silt loam (fine-silty, mixed, active, thermic Ultic Hapludalf). Soil pH and fertilizer applications are provided in Table 14. Vapor pressure deficit (VPD) was calculated based on temperature and relative humidity measurements recorded every five minutes with a data logger in each main plot (Fig. 8).

For evaluating the stomatal conductance recovery, two plastic covered, portable rainout-shelters with open ends were moved over the soybean plots to exclude precipitation and impose the water deficit stress in each year. Soil water content in the plant row was logged continuously at a depth of 45 cm in two of the water deficit subplots using Teros 21, a soil water potential sensor and 10HS, a volumetric water content soil moisture sensor (Meter Group, Pullman, WA).

Due to the portable nature of the rainout shelters, the size necessitated that the four subplot blocks be arranged with two blocks under each shelter. The frame of the rain-out shelters was aluminum and shaped like a gable and covered with 0.15 mm thick polyethylene (Atlas Manufacturing, Atlanta, GA). The shelters were moved over the plots when canopy coverage had reached around 80 percent on June 25, 50 days after planting (DAP), and on July 6, 33 DAP, in 2019 and 2020, respectively. In both years, recovery water supplement of 38 mm was applied at 127 DAP (2019) and 97 DAP (2020) when the majority of test plots had reached a state of moderate to severe wilting (visual rating 3-4). At this point, the plants had reached Stage III water deficit stress where NTR was estimated to be less than or equal to 0.10. The shelters were removed before harvest at 149 DAP (2019) and 135 DAP (2020). The center two rows of each plot were harvested with a plot combine equipped with weighing system and moisture meter. Plots weights were adjusted to 13% moisture content in order to calculate yields.

Data Collection

Stomatal conductance (g_s)

Stomatal conductance (gs, mol $H_2O/m^2/s^1$) was measured using a LiCor 6400 XT portable photosynthesis machine (Licor Biosciences, Lincoln, Nebraska). Measurements were taken in every subplot on one upper most fully developed leaf from the middle two rows on sunny days between 1100 and 1400 CST. To measure g_s, a leaflet segment of 6 cm^2 was enclosed in the LI-6400 leaf chamber. Calibration of the LI-6400XT followed the procedure described by Rosas-Anderson et al. (2014) where the chamber maintained ambient temperature through a constant-block-temperature feature, the chamber was set to expose the leaf to 2000 µmol photosynthetically active radiation (PAR) m^{-2} s^{-1}, and CO_2 concentration in the chamber was held at 400 µmol CO_2 mol^{-1} air. Stomatal conductance (g_s) was measured in the water deficit plots, under the rainout shelters at 80, 87, 98, 127, 128, and 134 DAP in 2019, and at 55, 63, 75, 82, 95, 98, 99, and 104 DAP in 2020.

Cumulative stress days (CSD), defined as the number of days since the imposition of the rainout shelters over soybean plots, describes the amount of time under which plants were exposed to a continuous exclusion of rainfall and irrigation in each year. For water deficit plots, g_s measurements began near the onset of water deficit stress: at 30 CSD in 2019 and 22 CSD in 2020. The recovery irrigation was applied at 77 CSD in 2019 and 64 CSD in 2020 and final g_s measurements were taken at 84 and 71 CSD in 2019 and 2020, respectively.

A g_s ratio was calculated to compare how the stomatal conductance in each plot changed as the soil water deficit increased over the season and then decreased as a result of the recovery irrigation, similar to the normalized transpiration rate used in controlled environment experiments. To calculate the g_s ratio, the g_s for each water deficit plot was divided by the g_s of that same plot on the first day of measurements (30 CSD in 2019 and 22 CSD in 2020). The g_s ratio was calculated for the last measurement day before recovery irrigation was applied, 77 CSD in 2019 and 62 CSD in 2020, and for the 1-day and 7-day recovery periods for both years.

Specific Leaf Area (SLA) and Visual-Wilting Score (WS)

During the process of measuring g_s, the leaves used for gas exchange measurement were destructively sampled and used to calculate specific leaf area (SLA) according to equation (2):

SLA = LA/DW (leaf area/leaf mass)

where LA was the leaf surface area and DW was the dry weight of the leaf.

Leaves were collected from the middle two rows of each plot between 1100 and 1400 CST on sunny days. The leaf sample selected from each plot was the youngest fully expanded leaf. The leaf was removed at the petiole and placed in a sealed plastic bag into which the sampler would blow in order to maintain humidity. The humidified bags were then placed on ice in the field to ensure that the samples remained fresh and turgidity was maintained. Leaf area was then measured using a Li-Cor LI-3100C Area Meter (Li-cor Biosciences, Lincoln, Nebraska). Then leaves were dried in an oven at 60^{o} C for 12 hours before obtaining the dry weight. In water deficit plots, additional leaf samples were collected at 107, 116, 121, 126, and 129 DAP in 2019 and at 52 and 68 DAP in 2020. The change in SLA over the recovery period was expressed as Δ SLA and was calculated by subtracting the SLA value for each plot on the last measurement before recovery from the value after recovery.

Water deficit plots were also visually rated using a rating scale of 0 to 5: 0 = no wilting, 1 = a few leaves wilted, 2 = half of leaves wilted, 3 = most leaves wilted, 4 = severe wilting, 5 = plant dead. Ratings were based on the condition of plants in the middle two rows of each plot and were conducted between 1100 and 1400 CST on sunny days. Each water deficit plot was visually rated on 80, 87, 88, 98, 101, 102, 107, 115, 116, 121, 126, 128, and 134 DAP in 2019 and on 43, 52, 55, 63, 68, 72, 75, 80, 81, 95, 96, 98, and 105 DAP in 2020. Rainout plots were visually rated on the same day that gas exchange measurements were collected with additional measurements collected at 88, 101, 102, 107, 115, 116, 121, and 126 DAP in 2019 and 43, 52, 68, 83, and 96 DAP in 2020.

Canopy Temperature (CT)

A thermal infrared camera, ICI 8640P (Infrared Cameras Inc., Beaumont, TX) was mounted to a handheld boom and two images were captured above the middle two rows of each water deficit plot at a height of 1.0 to 1.5 m above the plant canopy between 1100 and 1400 CST, one day before recovery irrigation, at 63 CSD, and one day after recovery irrigation in 2020. The ICI 8604P camera was chosen because it offered high sensitivity and accuracy while operating at a low (<1 W) power rate and compact size/weight (74.5 g), with a claimed accuracy of (+/−) 1 °C and Noise Equivalent Temperature Difference (NETD) thermal sensitivity of 0.02 °C.

Calibration of the radiometric JPEG images was conducted in IR-Flash Pro software (Infrared Cameras Inc., Beaumont, TX). The IR-Flash software provides batch processing for multiple images and then applies radiometric calibration using an internally installed factory calibration process. Then, the calibrated TIFF images were processed in ArcMap 10.8.1 (Esri, Redlands, CA). Extraneous pixels which included objects such as irrigation piping and large patches of bare soil were first clipped from the images manually before using the Iso Cluster Unsupervised Classification Tool in ArcMap to classify the thermal pixels in each image into 10 temperature classes.

The classified image was compared visually with original TIFF and classes were assigned to one of two categories: (1) canopy and (2) soil. The Zonal Statistics Tool within the Spatial Analyst Toolbar was then used to generate descriptive statistics, including the mean, of the pixels within each category for every image. The mean temperature of the canopy in images from the pre-recovery condition were subtracted from the means of the corresponding images in the post-recovery condition to calculate a change in canopy temperature (Δ CT) for each plot, which were then averaged to find the Δ CT for each genotype.

Statistical Analysis

For visual wilting score, results were separated by year and pre-recovery (defined as within the period seven days before recovery irrigation was applied), 1-day, 7-day (2019), and 8-day (2020) recovery ratings were averaged for each genotype. A mixed model analysis of variance (ANOVA) was conducted to determine if there was a significant difference in years and between genotypes before and after recovery. Mean separation letters were generated using Tukey's HSD. Specific leaf area (SLA) was averaged for each genotype from the time when CSD > 40 until recovery through irrigation application and SLA response to recovery irrigation was measured by the change (Δ SLA) in SLA from the last measurement day before recovery to the 2-day and 7-day recovery for each genotype. Genotype averages were compared in a mixed model ANOVA; mean separation for the water deficit stress condition was conducted using Tukey's HSD test while a Student's T test was used for Δ SLA during recovery. Yield and Δ CT were analyzed with a mixed model ANOVA and mean separation conducted with Tukey's HSD (p=0.05). A standard error of the mean (SEM) was calculated for each g_s ratio for the purpose of comparing the means.

Results

Visual Wilting Score

As soybean plants reached Stage III of water deficit stress, in the week leading up to application of recovery irrigation, differences in magnitude of visual wilting rating were observed among genotypes as well as a significant difference between years ($p<0.001$) (Table 6). In 2019, all but one of the genotypes, USG Allen, presented average visual ratings greater than or equal to 3, meaning that the plants were all significantly to severely wilted. However, in 2020, only two of the genotypes, RIL #1360 and TN08-101, presented visual ratings greater than 3, indicating that severity of wilting before recovery irrigation was less in 2020. Only two genotypes, Ellis and TN16-520R1, maintained low wilting scores during both 2019 and 2020 (Table 6).

In 2019, wilting scores increased after recovery irrigation in all genotypes, while in 2020, wilting scores decreased after recovery irrigation (Table 7). There were significant differences among genotypes for 1-day recovery in wilting score in 2019 ($p<0.01$) and 2020 ($p<0.01$). In 2019, USG Allen had the lowest wilting score at 2.9, and was significantly different from TN09-008, Ellis, TN08-101, TN Exp TN13-4508R2, and TN09-029, which ranged from 4.1 to 4.9. The remaining genotypes, TN16-520R1, USG 7496XTS, and RIL #1360 had wilting scores of 3.8, 4, and 4, respectively, but were not significantly different from any others.

In 2020, severity of wilting decreased for all genotypes within 24 hours of recovery irrigation and then a mixed response was observed between 1-day and week-long recovery with wilting severity increasing slightly in some genotypes. The genotype USG Allen, while the least wilted after 24-hr recovery in 2019, was the most wilted in 2020 at the 24-hr mark, although it had significantly recovered after a week. Ellis and TN09-029 were the least wilted in 2020 after recovery. In 2020, 1-day recovery resulted in more variation between genotypes than in 2019;

Ellis had the lowest wilting score at 0.85 and was significantly different than USG Allen at 2.83, TN08-101 at 2.50, and RIL #1360 at 2.49. TN09-029 had the second lowest wilting score (1.02) and was significantly different than the USG Allen and TN08-101. The differences observed among genotypes in the 7-day recovery (2019) and 8-day recovery (2020) were not statistically significant, which indicates that between 1-day and after a week of recovery, the differences in response among genotypes had approached an equilibrium.

Specific Leaf Area

After 40 cumulative stress days (CSD), SLA differed between genotypes in both 2019 and 2020. The average genotype SLA during the second half of the of the stress period, measured between 48 and 76 CSD in 2019, ranged from 230.4 cm^2g^{-1} (USG 7496 XTS) to 352.2 cm^2g^{-1} (TN09-029) (Table 8). USG 7496XTS had the lowest SLA in 2019 during the stress period and was significantly different from all other genotypes except Ellis (304.5 cm^2g^{-1}) and TN Exp TN13-4508R2 (295.0 cm^2g^{-1}) (Table 8). In 2020, during the period of stress when CSD was greater than 40 until recovery, SLA was measured from 42-63 CSD. Contrary to what was observed in 2019, USG 7496XTS had the highest SLA at 214.0 cm^2g^{-1} and was statistically different than only the genotype with the lowest SLA, TN16-520R1 at 166.6 cm^2g^{-1}. Specific leaf area values were much lower in 2020 than 2019 with the maximum in 2020 less than the minimum value in 2019.

For SLA averaged over the period where water deficit stress was developing in the plots (>40 CSD) genotypes behaved differently within years (Table 8). Significant differences in Δ SLA between genotypes were observed for 7-day recovery (Table 9) but not for 48-hour recovery in both years (data not shown). While variation was observed in SLA, no significant genotypic differences were detected with a mixed model ANOVA (p=0.05). However, a T-test showed that in 2019, the genotype with the greatest increase in SLA over the 7-day recovery period was Ellis (45.99 cm^2g^{-1}) which was significantly different from the lowest, USG 7496XTS (-94.81 cm^2g^{-1}) (Table 9). Genotypes TN08-101 (-9.41 cm^2g^{-1}) and TN Exp TN4508R2 (-50.27 cm^2g^{-1}) were the only two genotypes that were not statistically different than USG 7496XTS with those three constituting the only genotypes which decreased in SLA. In 2020, all genotypes expressed an increase in SLA, with RIL #1360 (69.19 cm^2g^{-1}) and TN16-520R1 (64.00 cm^2g^{-1}) being the two highest and only genotypes with a statistically significant different Δ SLA than the two lowest, USG Allen (17.25 cm^2g^{-1}) and TN08-101 (14.28 cm^2g^{-1}) (Table 9).

Stomatal Conductance

In 2019, g_s ratio at Stage III water deficit stress varied among genotypes and ranged from a low of 0.57 in USG 7496XTS to 2.21 in RIL #1360 (Table 10). Additionally, both Ellis and TN09-008 had g_s ratios greater than 1.0 at 77 CSD. In 2020, g_s ratio at peak stress was below 1.0 in all genotypes, ranging from a high of 0.91 in Ellis to a low of 0.09 in USG Allen (Table 10).

The stomatal conductance (g_s) ratio for the 1-day recovery period, the g_s 24 hours after recovery irrigation divided by the g_s of the same plots early in the season, trended differently in 2019 compared to 2020 (Table 10). In 2019, all genotypes g_s ratios for 1-day recovery compared to g_s ratio at Stage III stress decreased slightly (Table 10). However, in 2020, most genotypes had slightly higher g_s ratios for the 1-day recovery compared to Stage III stress (Table 10).

In 2019, the values of genotypes g_s ratios for 1-day recovery ranged from a high of 1.37 in RIL #1360 to a low of 0.20 in USG 7496XTS. Whereas, the g_s ratios for 1-day recovery in 2020 were all below 1.0 and ranged from a high of 0.74 in Ellis to a low of 0.18 in USG Allen (Table 10).

In 2019, genotype Ellis g_s ratio for 7-day recovery ranked as the highest with a value of 1.32 and TN08-101 was observed to have the lowest g_s ratio at 0.24. Similarly, in 2020, a wide range of g_s ratio responses was observed among genotypes between the 7-day and 1-day recovery period with certain genotypes, with Ellis (0.84), USG 7496XTS (0.49), and USG Allen (0.24) increasing slightly and the remaining genotypes decreasing slightly (Table 10).

Canopy Temperature

In 2020, most genotypes expressed a decrease in canopy temperature (CT) after recovery irrigation (Table 11). The largest decreases were observed in TN09-029 (-2.51 ^oC), Ellis (-2.38 ^oC), TN09-008 (-1.84 ^oC), and TN08-101 (-1.58 ^oC) (Table 11). USG Allen and USG 7496XTS had slight decreases in Δ CT of -0.79 ^oC and -0.28 ^oC, respectively, while genotypes TN16-520R1 and TN Exp

TN13-4508R2 had increases in ∆ CT (°C) of 0.65 °C and 1.19 °C, respectively (Table 11).

Yield

A mixed model analysis of variance (ANOVA) was conducted and determined that the year effect was not significant for yield therefore, the yield data for both years were combined then analyzed together. Significant yield variation among nine tested soybean genotypes was observed ($p=0.0002$) (Table 12). Genotype Ellis had the highest yield at 3516.56 kg/ha which was significantly higher than TN09-029 (2614.72 kg/ha), USG 7496XTS (2503.08 kg/ha), RIL #1360 (2468.11 kg/ha), and TN Exp TN13-4508R2 (2378.0 kg/ha), according to Tukey's HSD. Genotype Ellis yielded 25.6, 28.9, 29.8, and 32.3 (%) higher than TN09-029, USG 7496XTS, RIL #1360, and TN Exp TN13-4508R2, respectively (Table 12).

Discussion

For crop productivity under drought conditions, the recovery from severe periods of drought stress such as Stage III water deficit stress as described in this study, is critical. While the physiological mechanisms involved in crop recovery from Stage III water deficit stress are still being studied, the necessary research for identifying adapted genotypes with desirable survival traits is lacking. A few studies have looked at physiological recovery of transpiration rate from short term drought stress (Cerezini et al., 2016; Rosas-Anderson et al.,

2020) with varying results. It has been a challenge, however, to overcome the limited genetic base of soybean and identify variation in water saving and water deficit recovery traits among soybean genotypes (Carter et al., 2004). The potential for soybean genotypes to recover from a more extended Stage III stress under field conditions remains undocumented.

Our current field study was particularly unique because of the application of simulated water deficit conditions with portable rainout shelters and the subsequent Stage III stress recovery. Genotypic differences among soybean genotypes were observed in the physiological parameters measured in this study and help to draw associations with the observations gathered in the controlled environmental studies.

One important consideration for the synthesis and discussion of the results observed in the two years of this field study are the environmental conditions during the growing season, in particular later in the season when the water deficit stress developed into Stage III, as well as during the recovery phase of the experiment. The average daily high temperature in September in 2019 was 33.4 ^{o}C compared to 28.1 ^{o}C in 2020 (Figure 7). The daily low temperatures also differed by about 2 ^{o}C in September 2019 and 2020. Also, vapor pressure deficit (VPD) was much greater in 2019, particularly after August 25, compared to 2020 (Figure 8).

Given the diverse range of TR responses to VPD levels for these soybean genotypes observed in our controlled environmental study and the fact that VPD in the field was often much higher than the maximum that was tested in the controlled environment (2.5 to 3.5 kPa) it can be expected that differences in TR responses and other physiological observations among years can be attributed to the difference in temperature and VPD (Shekoofa et al., 2016, 2020; Sinclair et al., 2017; Sheldon et al., 2021).

The difference between years in the range of visual wilting scores of genotypes during Stage III water deficit stress was approximately 1 on the 0-5 scale (Table 6). This could be attributed to two factors: (1) the higher VPD observed in 2019 resulted in generally greater incidence of wilting at midday when measurements were gathered and (2) in 2019, the rainout shelters were moved over the field 13 days sooner than in 2020, therefore, soybean genotypes were struggling with a longer period of Stage III water deficit stress. However, some patterns carried across both years; notably, Ellis and TN16-520R1 were in the top three of least wilted genotypes in both years under Stage III water deficit stress (Table 6).

When comparing visual wilting scores 1-day after recovery and again a week (7 days in 2019, 8 days in 2020) after recovery, there was significant genotypic variation at the 1-day mark and the absence of significant differences after a week (Table 7). This indicates that the difference in genotype ability to recover maintenance of leaves had reached equilibrium and stabilized before the week had elapsed. Rosas-Anderson et al. (2021) observed a similar finding where leaf expansion rate in soybean after recovery from Stage III water deficit stress stabilized within one or two days of rewatering. Again, the difference in years indicates that environmental conditions affected recovery as well; wilting scores in 2019 increased from the pre-recovery measurements to the 1-day recovery and again to week-long recovery, meaning that wilting continued to increase due to drought severity and high evaporative demand after recovery irrigation.

The Δ SLA was calculated to represent the change in SLA after recovery. Because of the assumption that no significant changes in leaf growth or morphology had occurred in 24 hours, the 7-day recovery period was used to compare the differences among genotypes (Table 9). Previous studies have shown that specific leaf area tends to decrease in grain legumes due to water deficit stress (Pandey et al., 1984; Turk & Hall, 1980), possibly leading to greater water conservation due to the smaller surface to volume ratio (Lopez et al., 1997). Shekoofa et al. (2016) reported this same observation as a negative correlation between water use efficiency coefficient (WUE_k) and SLA. In the current study, increases in SLA 7 days after recovery in most genotypes are consistent with other reports. However, the longer water deficit period imposed in 2019 as compared to 2020 may have led to the greater magnitude of genotype variation in Δ SLA. Genotypes which had a negative Δ SLA in 2019 did not show the predicted increase in SLA after recovery.

Stomatal conductance ($g_{s)}$ is a measure of transpiration, therefore, on the days which genotype g_s responses were measured environmental conditions were closely monitored; the VPD on the days for which g_s measurements are presented can be found in Table 13. In general, a pattern across both years indicates that on the last g_s measurement before recovery, at Stage III water deficit stress, both Ellis and TN09-008 consistently ranked among the highest in g_s ratio (Table 10) which indicates that there was possibly more water available for them in the soil, considering the potential for soil water conservation traits, especially in Ellis, that have been previously identified (Shekoofa et al., 2018).

Stomatal conductance ratio rates after recovery showed a much higher range of variability among genotypes in 2019, with several genotypes expressing ratios greater than 1.0 on both days. In 2019, vapor pressure deficit (VPD) on the 1-day and 7-day recovery measurement was 4.25 kPa and 5.16 kPa, respectively, compared to 2.79 kPa and 2.88 kPa in 2020 (Table 13). This difference in VPD likely resulted in some genotypes expressing the safety mechanism which allows for reduced control of transpiration at high temperature and VPD, even in genotypes with the limited transpiration trait in high VPD (2.5 to 3.5 kPa) conditions. (Sheldon et al., 2021; Shekoofa et al., 2016; Seversike et al., 2013; Sermons et al., 2012; Yang et al., 2012). Shekoofa et al. (2016) described this safety mechanism as a response to frequently occurring high temperatures that could result in heat stress, stating that it may be advantageous to consider cultivars that lose the limited transpiration trait at high temperature (i.e., 38°C) and VPD rather than 32°C or other temperatures below 38°C.

Temperature and evaporative demand are important environmental variables that impact plant physiological parameters such as stomatal conductance (g_s), transpiration, and leaf maintenance. Under well-watered conditions, increasing temperatures can cause rising g_s and enhance the evaporative cooling of transpiring leaves (Urban et al., 2017). However, water deficit stress reduces leaf g_s and transpiration rate and, consequently, increases the canopy temperature (Sagan and Fishman, 2018).

At the 1-day recovery mark, three of the genotypes which expressed a reduction in canopy temperature greater than 1.0 °C, Ellis, TN09-029, and TN08-101, which ranked first, second, and fourth in magnitude of reduction in 2020 Δ CT (Table 11), ranked first, second, and third in g_s ratio on the same day (62 CSD) (Table 10).

This association of CT reduction after recovery in 2020 with g_s helps to further the possibility of thermal imaging as a plant phenotyping tool for drought response. This is strengthened by an association with wilting score; Ellis and TN09-029, expressing the largest CT reduction, also scored the lowest for wilting on the same day, joined by TN09-008 with the third greatest in CT reduction and one of only five genotypes with a wilting score of less than 2.0 on the same day (Table 7). Bai and Purcell (2018) observed an interaction between CT and slow and fast-wilting genotypes where slow-wilting genotypes had a lower canopy temperature during water deficit stress. The low canopy temperature was also correlated with an increase in yield. Additionally, a study which identified genomic regions associated with canopy temperature in diverse soybean genotypes identified fifteen chromosomal regions where associations with CT and canopy wilting were coincident (Kaler et al., 2018).

Ultimately, it is the combination of various physiological traits that provide a soybean plant the ability to both function sustainably under a water deficit stress and utilize resources quickly when they are available. Yield provides a way to observe how the set of phenotypic characteristics of a genotype will affect the performance of that genotype under applied conditions. Average genotype yields over both seasons help to confirm what the results from observing each individual trait imply. For example, Sinclair et al. (2018) identified an association in peanut genotypes of early transpiration decrease, delayed wilting, and increased yield under a similar rainout shelter experiment which was attributed to soil water conservation. Indeed, in our experiments, the top five yielding genotypes (Table 12) are all among those which have expressed desirable responses in several of the individual water saving traits.

Our field studies complemented the results found and reported in both controlled environment experiments. The physiological mechanisms of transpiration control and leaf maintenance described earlier helped to identify genetic variation in plant recovery from Stage III water deficit stress and can provide the best resource for plant breeders to develop and release drought tolerant soybean cultivars. Overall, Ellis consistently expressed a desirable response to water deficit conditions in the field experiments, confirming what was observed in the controlled environmental studies. During Stage III stress Ellis exhibited a much lower wilting severity as well as superior recovery of transpiration and leaf maintenance after re-watering. These responses and the increased yield of Ellis relative to other genotypes under the water deficit condition make it a strong candidate for use in breeding programs and production for water-limited environments.

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Appendix

Table 5. 2019 and 2020 rainfall and irrigation amount by month.

		Rainfall (May-October) (mm)	
		2019	**2020**
Month	**May**	82.55	101.09
	June	114.81	56.9
	July	288.54	106.7
	August	132.08	107.95
	September	36.58	78.99
	October	141.48	28.19
		Rainfall and Irrigation per Treatment (mm)	
Treatments	**Irrigated***	76.2	114.3
	Rain-out Shelters**	127.1	159.5

*Indicates total amount of irrigation applied (does not include rainfall received)

** Represents rainfall received from planting until rainout shelters were moved over plots in June. At R5.5 soybean plants under the rainout shelters received an additional 38 mm of irrigation (water deficit stress-recovery treatment).

Table 6. Visual wilting score for genotypes during the week prior to application of recovery irrigation. Genotypes followed by the same letter are not significantly different (Tukey's HSD).

2019 (71 & 76 CSD*)			2020 (62 & 63 CSD*)		
Genotype	**Mean WS**		**Genotype**	**Mean WS**	
TN09-029	3.94	a	**RIL #1360**	3.50	a
TN Exp TN13-4508R2	3.75	a	**TN08-101**	3.25	ab
TN09-008	3.75	a	**USG Allen**	2.92	abc
USG 7496XTS	3.69	a	**TN Exp TN13-4508R2**	2.83	abc
RIL #1360	3.63	a	**TN09-008**	2.75	abcd
TN08-101	3.44	ab	**USG 7496XTS**	2.58	bcd
Ellis	3.31	ab	**TN16-520R1**	2.31	cd
TN16-520R1	3.00	ab	**Ellis**	2.25	cd
USG Allen	2.19	b	**TN09-029**	2.00	d

*Cumulative Stress Days

Table 7. Mean genotype wilting score 1 day, 7 (2019), and 8 (2020) days after application of recovery irrigation in 2019 and 2020. Genotypes followed by the same letter are not significantly different (Tukey's HSD).

2019					
1-day Recovery			**7-day Recovery**		
Genotype	**Mean WS**		**Genotype**	**Mean WS**	
TN09-029	4.88	a	**TN09-029**	5.00	a
TN Exp TN13-4508R2	4.38	a	**USG 7496XTS**	4.63	a
TN08-101	4.25	a	**Ellis**	4.50	a
Ellis	4.13	a	**TN16-520R1**	4.50	a
TN09-008	4.13	a	**TN Exp TN13-4508R2**	4.38	a
RIL #1360	4.00	ab	**TN08-101**	4.38	a
USG 7496XTS	4.00	ab	**TN09-008**	4.38	a
TN16-520R1	3.88	ab	**USG Allen**	4.13	a
USG Allen	2.88	b	**RIL #1360**	4.13	a
p-value	0.0009		**p value**	0.2	

2020					
1-day Recovery			**8-day Recovery**		
USG Allen	2.83	a	**TN08-101**	2.38	a
TN08-101	2.50	a	**RIL #1360**	2.22	a
RIL #1360	2.49	ab	**TN09-008**	2.19	a
TN Exp TN13-4508R2	2.16	abc	**TN16-520R1**	2.13	a
TN09-008	1.99	abc	**TN Exp TN13-4508R2**	2.02	a
TN16-520R1	1.88	abc	**USG 7496XTS**	2.02	a
USG 7496XTS	1.33	abc	**USG Allen**	1.86	a
TN09-029	1.02	bc	**TN09-029**	1.16	a
Ellis	0.85	c	**Ellis**	1.16	a
p-value	0.003		**p-value**	0.053	

Table 8. Mean specific leaf area (SLA) for each genotype during the period of stress after cumulative stress days were greater than 40 and before recovery irrigation. Genotypes followed by same letter are not significantly different (Tukey's HSD).

2019 (48-76 CSD)			2020 (42-63 CSD)		
Genotype	**SLA (cm^2g^{-1})**		**Genotype**	**SLA (cm^2g^{-1})**	
TN09-029	352.25	a	**USG 7496XTS**	214.0	a
TN08-101	323.44	a	**TN08-101**	203.06	ab
RIL #1360	317.11	a	**TN Exp TN13-4508R2**	197.90	ab
USG Allen	315.31	a	**USG Allen**	188.97	ab
TN16-520R1	307.19	a	**TN09-008**	182.87	ab
TN09-008	306.06	a	**RIL #1360**	182.28	ab
Ellis	304.46	ab	**Ellis**	178.87	ab
TN Exp TN13-4508R2	294.96	ab	**TN09-029**	178.53	ab
USG 7496XTS	230.38	b	**TN16-520R1**	166.64	b

Table 9. Change in specific leaf area from last measurement point before recovery irrigation to 7 days after recovery irrigation. P-values show significance of differences among genotype. Genotypes followed by the same letter are not statistically different (Student's T test).

2019			2020		
Genotype	**Δ SLA (cm^2g^{-1})**		**Genotype**	**Δ SLA (cm^2g^{-1})**	
Ellis	45.99	a	**RIL #1360**	69.19	a
USG Allen	45.99	a	**TN16-520R1**	64.00	a
TN09-008	42.02	a	**TN09-029**	54.78	ab
TN09-029	18.90	a	**TN09-008**	40.59	ab
TN16-520R1	17.45	a	**USG 7496XTS**	38.00	ab
RIL #1360	17.42	a	**TN Exp TN13-4508R2**	24.73	ab
TN08-101	-9.41	ab	**Ellis**	20.46	ab
TN Exp TN13-4508R2	-50.27	ab	**USG Allen**	17.25	b
USG 7496XTS	-94.81	b	**TN08-101**	14.28	b
p-value	0.059		**p-value**	0.15	

Table 10. Stomatal conductance (gs) ratios for genotypes in 2019 and 2020. Standard error of the mean (SEM) is presented for each ratio on each day.

	2019						2020					
	77 CSD		1-day		7-day		62 CSD		1-day		7-day	
Genotype	g_s ratio	SEM	g_s ratio	SEM	g_s ratio	SEM	g_s ratio	SEM	g_s ratio	SEM	g_s ratio	SEM
Ellis	1.50	0.61	0.85	0.44	1.32	0.51	0.91	0.64	0.74	0.39	0.84	0.50
RIL #1360	2.21	1.12	1.37	0.67	1.22	0.67	0.31	0.13	0.73	0.07	0.54	0.17
TN EXP TN13-4508R	0.85	0.23	0.87	0.47	0.62	0.22	0.40	0.22	0.68	0.14	0.53	0.12
TN08-101	0.95	0.28	0.58	0.18	0.24	0.15	0.14	0.04	0.61	0.10	0.52	0.23
TN09-008	1.47	0.59	1.04	0.32	1.04	0.42	0.57	0.25	0.59	0.30	0.52	0.31
TN16-520R1	0.82	0.23	0.56	0.15	0.79	0.20	0.69	0.03	0.49	0.22	0.51	0.25
USG 7469XTS	0.57	0.05	0.20	0.05	0.26	0.05	0.36	0.07	0.43	0.20	0.49	0.20
USG Allen	0.79	0.33	0.45	0.15	0.50	0.22	0.38	0.20	0.24	0.04	0.24	0.14
TN09-029*	-	-	-	-	-	-	0.69	0.03	0.73	0.07	0.54	0.17

*The genotype TN09-029 was excluded from g_s recovery observations in 2019 because it had senesced before recovery irrigation was applied.

Table 11. Change in 2020 canopy temperature (CT) from 24 hrs pre-recovery to 24 hrs post-recovery. Means followed by the same letter are not significantly different (Tukey's HSD, p=0.05).

Genotype	**Δ CT (ºC)**	
TN Exp TN13-4508R2	1.19	a
TN16-520R1	0.65	ab
RIL #1360	0.45	ab
USG 7496XTS	-0.28	ab
USG Allen	-0.79	ab
TN08-101	-1.58	ab
TN09-008	-1.84	ab
Ellis	-2.38	ab
TN09-029	-2.51	b
p-value	0.007	

Table 12. Yield in water deficit plots averaged for 2019-2020. Means followed by the same letter do not differ significantly at p=0.05 (Tukey's HSD)*.

Genotype	Yield (kg/ha)	
Ellis	3516.56	a
TN16-520R1	3256.97	ab
USG Allen	3238.1	abc
TN08-101	3232.76	ab
TN09-008	3015.54	abc
TN09-029	2614.72	bc
USG 7496XTS	2503.08	bc
RIL #1360	2468.11	bc
TN Exp TN13-4508R2	2378.0	c

*A mixed model analysis of variance (ANOVA) was conducted and determined that the year effect was not significant for yield therefore, the yield data for both years were combined.

Table 13. Mean vapor pressure deficit (VPD) in the plant canopy during collection of stomatal conductance measurements**.**

2019		2020	
Day	**VPD (kPa)**	**Day**	**VPD (kPa)**
30 CSD	3.19	**22 CSD**	2.44
77 CSD	2.71	**62 CSD**	3.53
24-hr	4.25	**24-hr**	2.79
7-day	5.16	**7-day**	2.88

Table 14. Soil pH and base fertilizer applications in field plots

Soil characteristics	2019	2020
soil pH	6.7	6.6
phosphorous (kg/ha)	33.6	33.6
potassium (kg/ha)	89.7	112.1
sulfur (kg/ha)	13.5	16.8

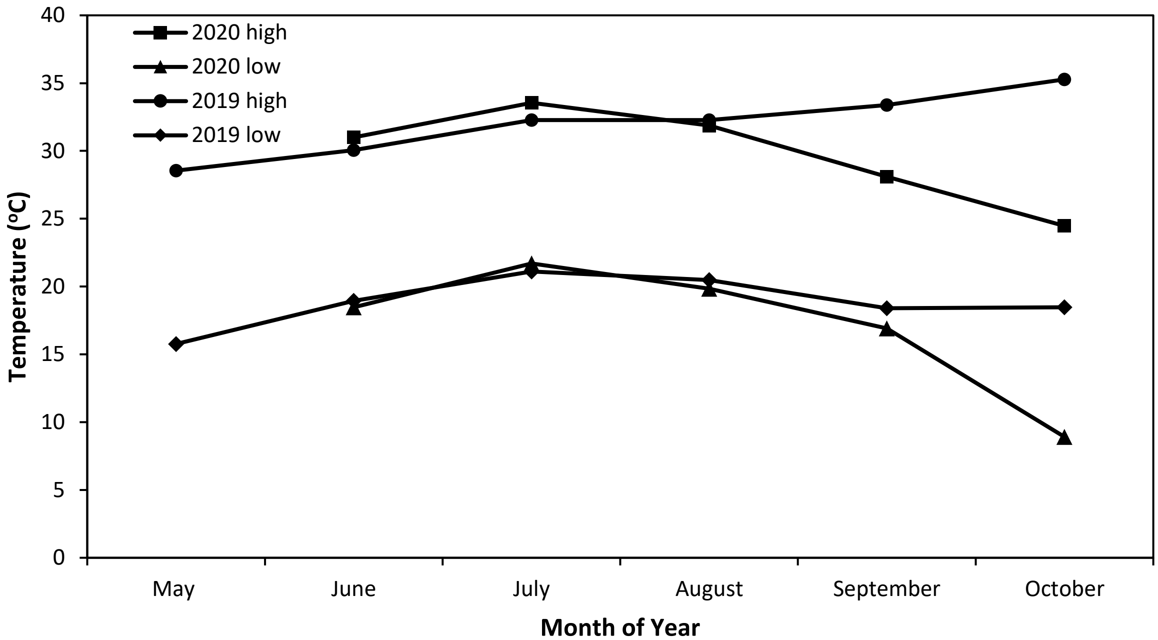

Figure 6. Mean monthly high and low temperatures for both 2019 and 2020 at the West Tennessee Research and Education Center during the period from planting to harvest.

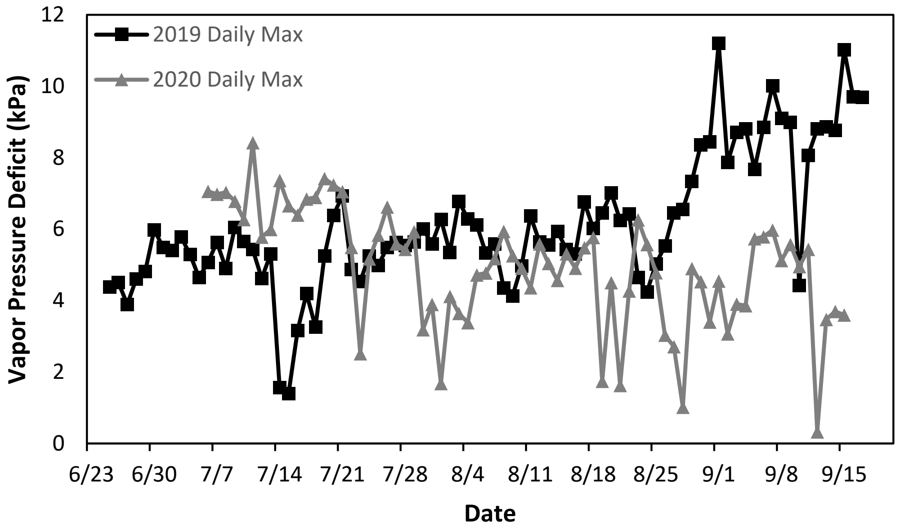

Figure 7. Daily maximum vapor pressure deficit (kPa) during the field experiment in both 2019 and 2020. The date range represents the period during which the plots were covered with portable rainout shelters until 7 days after recovery irrigation was applied.

CHAPTER IV CONCLUSION

Discussion and Conclusions

In the United States, the sensitivity of soybean yield to drought will continue to increase (Zipper et al., 2016). With only six percent of U.S. soybean production grown under irrigation (Irwin et al., 2017), water deficit stress is a critical environmental factor affecting dryland soybean production (Jumrani & Bhatia, 2018). In response, breeding efforts for the development of drought tolerance in soybean have focused on empirically selecting genotypes with higher yields under dry conditions with limited understanding of the underlying physiological traits (Devi et al., 2014). Indeed, the complexity of interactions among physiological traits in plants make the isolation and study of individual traits and genes difficult (Sinclair and Purcell, 2005), necessitating the use of new tools for phenotyping under applied field conditions (Sinclair, 2011).

This study sought to evaluate soybean genotypes developed for production in the Mid-South region of the United States for several traits which have been linked to drought resistance, using associations established in previous studies as well as new techniques. Among these are the delayed-wilting response to soil drying first identified in PI 416937 (Sloane et al., 1990) which has been linked to limited transpiration under high vapor pressure deficit (VPD) (Fletcher et al., 2007) and early stomatal closure under soil drying (Shekoofa et al., 2021). Most importantly, this study investigated the potential for genetic variability in soybean in the ability to recover from water deficit stress by measuring leaf-scale responses such as wilting and specific leaf area (SLA) as well as gas exchange and canopy temperature during the period immediately after re-wetting. Genotypic variation in transpiration rate (TR) recovery from water deficit stress has been identified in peanut (Rosas-Anderson et al., 2014), cowpea (Manandhar et al., 2017), and most recently in several North Carolina soybean genotypes (Rosas-Anderson et al., 2021; Rosas-Anderson et al., 2020).

In the controlled environment studies presented in Chapter 2, experiment 1 examined leaf wilting rate under low fraction of transpirable soil water (FTSW) (<0.30) and normalized transpiration rate (NTR) and leaf wilting recovery from Stage III stress. The most significant finding in this study linked the slow-wilting trait observed in four out of eight genotypes to a higher rate of NTR recovery after Stage III stress. Furthermore, the same genotypes which expressed slow-wilting and superior recovery in this study were associated with early stomatal closure under soil drying (Shekoofa et al., 2021). Since slow-wilting has been linked to several other drought tolerance traits (Devi et al., 2014), this supports the use of wilting severity as a phenotyping tool in drought tolerance assessments including ability for recovery from drought stress. In experiment 2, two transpiration rate (TR) responses for increased VPD were observed: a linear response and a breakpoint (BP) response which signified a limitation in maximizing TR (i.e., limited TR trait) at a specific VPD. Almost all genotypes expressing the BP response to increased VPD had desirable responses in the traits measured in experiment 1 and the opposite was true for genotypes with the linear response. In these experiments, the association of superior recovery in genotypes like Ellis, USG Allen, and TN09-029 with water-saving traits such as limited TR at high VPD (Sinclair et al., 2010) and early stomatal closure under soil drying (Shekoofa et al., 2021) is promising in identifying an ensemble of drought tolerance traits.

In Chapter 3, observations gathered in the field helped support findings from the controlled environment studies in Chapter 2. However, much higher temperature and VPD in the field later during the growing season in 2019 likely contributed to differences in plant responses to water deficit stress among the two years. Most likely, plant response to increased VPD, especially the safety mechanism which allows for resumption of high TR after VPD reaches a certain threshold in plants with the limited TR trait (Shekoofa et al., 2016), can give context to these findings. For example, a high recovery g_s ratio in Ellis in both years indicates that a VPD safety mechanism in Ellis, when very high temperatures and VPD are frequent may have allowed it continue to transpire even in 2019 when daily VPD was reaching maximum values (Fig. 8) , while the VPD BP for Ellis was 2.7 kPa. In contrast, a very low g_s ratio after recovery in USG Allen indicates that it may have maintained limited TR even as VPD continued to increase, while the VPD BP for USG Allen was 2.1 in experiment 2. Despite environmental interactions among years, trends observed in the field confirmed the findings from the controlled environment. Genotypes with less wilting at Stage III in the field were the same as those in the slow-wilting group in the controlled environment and a high NTR recovery in the greenhouse was associated with a higher recovery g_s ratio in the field.

The genotypes Ellis, USG Allen, TN09-029, and TN16-520R1 had a desirable response in a majority of the traits tested, with Ellis representing the superior genotype overall, which was confirmed by the two-year yield data. This was confirmed across two seasons of field data and two controlled environment experiments. The combination of physiological traits may position these genotypes well as having greater potential for yield increase over a range of drought-stressed and high temperature environments.

In field studies of drought tolerance, the interaction of VPD, temperature, and soil water deficit plays a large role in plant physiological responses. While this interaction is complex, it raises the opportunity for further study of the increases in temperature and changes in rainfall patterns predicted to occur with the changing climate. Additionally, adopting practices such as canopy temperature in estimations of plant transpiration across plots and fields offers an opportunity to gain a much larger-scale perspective beyond the single plant observations used in these studies. In future studies, genotypes mentioned above which exemplified drought tolerance traits should be evaluated further. These may be especially desirable for drier environments where the slow wilting and the low threshold for the limited-transpiration trait (i.e., low VPD breakpoint) could contribute to superior yield performance. This could include larger scale field trials that allow observation under differing soil water and atmospheric conditions and incorporating physiological observations and traits into crop models.

Modelling of observed traits under various future climate scenarios and locations could also help predict how exactly the various plant responses will affect yield and production in the future. For plant breeders, incorporating germplasm from genotypes which expressed the best responses to drought tolerance in these studies provides an opportunity to develop cultivars with a greater potential for yield stability as the climate changes.

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VITA

Samuel Purdom was born in 1992 in Athens, OH. After graduating from Virginia Polytechnic Institute and State University in 2013 with a B.S. in Environmental Resource Management, Samuel worked for several years in the private land conservation field, before pursuing a lifelong dream to serve in the U.S. Peace Corps. He served for three years as an agriculture volunteer in Zambia from 2016-2019 which is what inspired his passion for agriculture and climate change research and led him to completing his M.S. in 2021 at University of Tennessee Knoxville in Plant Sciences.